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Bioremediation Studies on Pesticides in Soil by Novel Microorganisms Isolated from Agricultural Fields

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Abstract

Pesticides are organic compounds widely used in agriculture for increasing agricultural yield, soil productivity and to control the insect vectors for prevention of various plants, human and animal epidemics. Pesticides are widely distributed in our ecosystem. Organophosphates are found to be one of the most common group of pesticides found across the world. However, excessive and indiscriminate use of pesticides have generated a number of environmental problems such as release of toxic waste in the environment resulting to contamination of air, water and terrestrial ecosystem. In order to control the harmful effects of pesticides, multiple attempts have been made like the use of physical, chemical, biological and enzymatic approaches to reduce pesticides. However, physical and chemical methods have proven to be inefficient. One of the most promising field of biotechnology that offers a novel approach to degrade the pesticides is bioremediation. Bioremediation uses microorganisms, microbial or plants enzymes to detoxify contaminants in the soil and other environments. Three group of microorganisms i.e., bacteria, actinomycetes and fungi have been found to degrade the pesticides. Bacterial group involved in degradation includes Bacillus, Pseudomonas, Flavobacterium, Arthrobacter, Paracoccus, Aerobacter, Alcaligens, and Sphingomonas. Fungi having potential bioremediation capacities includes Fusarium, Aspergilus niger, Penicillium, Oxysporum. Streptomycetes have been found to degrade pesticides among the actinomycetes group. This review highlights various aspects of microbial degradation of pesticides including the types of pesticide degrading microbes and mechanism of degradation.

Keywords: *Pesticides, Organophosphates, Bioremediation, Microorganisms*

1. Introduction

Since the beginning of modern era, the traditional forms of agriculture has been inadequate to satisfy the needs of the human population on the earth, which has resulted in increased use of pesticides in agriculture. Pesticides are widely distributed in our ecosystem. According to the Food and Agriculture Organization of the United Nations (FAO), pesticide use increased by 36% from 2000 to 2019 (FAO World food and agriculture). Pesticides have become an indispensable part of modern agriculture. Pesticides are known to be the natural or man-made substances used to control pests, weeds, and diseases in plants in various agricultural activities. They include insecticides, herbicides, nematicides, fungicides, molluscicides, rodenticides, plant growth regulators [1] (Zhan et al., 2020; Bhatt et al., 2021; Zhang et al., 2021). The losses of crops caused by insect pests are quite high across the developed and developing countries (Dhaliwal et al., 2015) [2]. Pesticides are widely used as tool for plant protection and improvement of crops in the process of agricultural development. (Sharma et al.,2019) [31]. The most important benefits include increased crop yields, improved food safety, reduced labor and energy use (Cooper and Dobson,2007) [3]. For example, during the last few decades, the average crop yields in agriculture have steadily increased (Oerke, 2006). The intensity of protection for crops, has increased by 15-20-fold around the world, and has made agriculture more productive and profitable. However, despite the increased usage of pesticide, crop losses have still not decreased very significantly over the last few years. (Oerke, (2006) [4]. Pesticides are widely used in agriculture to prevent or control pests, diseases, weeds and other plant pathogens in order to reduce yield losses and maintain high product quality [30] (Abbasi and Krishnan, 1993). Pesticides are commonly used to protect animals from pests. Although pesticides have an important role in agriculture to solve the problem of feeding the world's over-growing population, the excessive and indiscriminate use of pesticides have generated a number of environmental problems. The pesticides enter into the ecosystem through water and air (Mali et al.,2019) and concentrate along the food chain and creates a potentially severe threat to biodiversity and human health (Sulamain et al.,2018) [5]. Even though many of the pesticides can degrade rapidly in the soil, some are persistent in nature and can be potentially hazardous as a direct result of accidental spills, runoff from application in agricultural areas, and discharge from containers and waste disposal systems (Cui et al., 2001) [6]. When pesticides are applied to crops, they reach the soil through rain, irrigation and wind. In addition to the above, several pesticides are ubiquitous compounds, which persist in soil and sediments due to less bioavailability [29] (Odukkathil and Vasudevan,2013). Therefore, pesticides are highly toxic and it can cause chronic abnormalities in humans, destroying the environment and biodiversity (Jariyal et al., 2015) [7,8]. Pesticides usage causes serious concern not only to potential effects on human health, but also on the impact on wildlife and our ecosystem [28] (Kamrin,1997). Pesticides effects on animal life such a fish, birds, reptiles, insects, mosquitoes etc., and which was a serious threat to biodiversity (Golden, 2007) [6]. The environmental problems caused by pesticide include decreased soil fertility, soil acidification, nitrate leaching, increased floral and faunal resistance, groundwater and surface water pollution, and contamination of soils (Kumari et al., 2008; Bishnu et al., 2009; Pujeri et al., 2010; Lari et al., 2014; Verma et al., 2014) [9]. Pesticides are found to be water soluble and are highly toxic. In order to control the harmful effects of pesticides, multiple attempts have been made like the use of physical, chemical, biological and enzymatic approaches to reduce the effects of pesticides. Some of the physical methods of degradation of pesticides are adsorption, zeolites, activated carbon and polymeric materials whereas some chemical methods include advanced oxygen processes, UV-H_2O_2 and UV-ozone, Fenton reaction and Zero valent iron etc. However, these methods are high in cost, low yield and can cause pollution to the environment. One of the most promising field that offers a novel approach to curb the problem is biotechnology. Biological remediation or bioremediation is an effective technology that helps in the complete breakdown of organic compounds into simpler and harmless end products (CO_2 and H_2O). It is low-cost and environmentally friendly as compared to physical or chemical methods (Nwankwegu and Onwosi 2017) [10]. Microorganisms are used for pesticide degradation because they possess enzymes that utilize the contaminants as a food. The aim of bioremediation is to provide optimum levels of nutrients essential for their metabolism. This will result in degradation of substances which are hazardous to environment. All metabolic reactions are controlled by enzymes. Many enzymes have shown a wide degradation capacity due to their nonspecific and specific substrate affinity (Kumar et al.,2011) [11]. Pesticide biodegradation is a novel and environmentally acceptable pesticide pollution control for a long-term environmental benefit. Microorganisms play a

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significant role in the breakdown of pesticides and are known for their uses in human welfare. Several recent studies have demonstrated the potential of microorganisms, isolated from sewage or soil to degrade pesticides. These microbes include several bacterial and fungal strains, actinomycetes, algae, etc. (Kafilzadeh et al., 2015) [12]. There are few methods of bioremediation with microorganisms: remediation through improved natural attenuation (using natural capacities of the microorganisms present in the matrix), bioaugmentation (introduction of genetically modified microorganisms), and biostimulation (addition of stimulators like electron acceptors and nutrients) (Helbling 2015; Nwankwegu and Onwosi,2017) [13]. Genetically modified organisms are the organisms whose genetic material have been altered and foreign gene is inserted by genetic engineering. Genetically engineered microorganisms (GEMs) have shown great potential for bioremediation applications in soil, groundwater, and activated sludge environments, exhibiting enhanced degradative capabilities for a wide range of chemical contaminants. GEMs have been effectively used for biodegradation purpose and represent a promising approach with broad implications in the future. The present review focuses on the microbial degradation of pesticides highlighting the chemistry of some important known pesticides and microorganisms involved in their degradation.

2. Chemistry of Pesticides

The chemistry of organic pesticide compounds has a vast literature which is dependent on the discipline studied. Pesticides are classified as following:

- On the basis of chemical nature, pesticides which are most commonly used worldwide are classified into two groups: organochlorines, organophosphates.
- On the basis of Application requirement (agriculture, public health, domestic).
- On the basis of Target organism or targeted use (insecticide, herbicide, fungicide, etc.)

2.1 Organochlorines

Organochlorines (OC) are a group of chlorinated compounds widely used as pesticides. Organochlorine pesticides are made up of hydrocarbons in a cyclic manner and listed out some of the examples in Table 1.

Table 1 Some Examples of Chemical Pesticides

S. No	Chemical Group	Chemical Nature	Chemical Name
1	Organoclorines	Chlorinated compounds	DDT, Dicofol, Eldrin, Dieldrin, Chlorobenziate, Lindane, BHC, Methoxychloro Aldrin, Chlordane, Heptaclor, Endosufan, Isodrin, Isobenzan, Toxaphene, Chloro propylate
2	Organophophates	Eaters or thiol derivatives of phosphoric acid	Dimefox,mipafox, methyl Parathion, Ronnel, enitrothion, Bidrin, Phorate, Fenthion, abate, demetox, malathion, diptrex, chlorpyriphos

These chemicals belong to the class of organic pollutants (POPs) with high persistence in the environment. Previously, OC insecticides were used in controlling malaria and typhus, but now they are banned in most of the advanced countries (Aktar et al., 2009). They have a low solubility in water and therefore tend to adsorb on to particles. OCPs are highly toxic, stable, and resistant to degradation in the environment. OCPs are less polar, least water soluble, have high lipid solvency and are stable towards deterioration. OCPs are less susceptible towards biodegradation (Gupta, 2004; FAO, 2005) [14]. They are released from agricultural pesticide application, waste dumping into landfills, and industrial discharge. Some of the most widely used organochlorine insecticides are DDT, hexachlorocyclohexane (HCH), aldrin and dieldrin (Lallas, 2001) [15].

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2.2 Organophosphates

Organophosphates are ester or thiol derivatives of phosphoric acid, as shown in Figure 1&2. They are coupled with two organic groups and an additional side chain consisting of cyanide, thiocyanate, or nerve impulses in the brain (Toole & Toole, 1995) [16,32]. After the transmission of nerve signal, acetylcholine gets hydrolysed by an enzyme called acetylcholine esterase. Acetylcholine esterase converts acetylcholine into choline and acetyl CoA by binding the substrate at its active site form a stable enzyme substrate complex. Further reactions involve release of choline from the complex and then rapid reaction of acylated enzymes with water to produce acetic acid and the regenerated acetylcholine esterase. Organophosphates inhibit the normal activity of the acetylcholine esterase by covalent bonding to the enzyme, thereby changing its structure and function. They bind to the serine 203 amino acid active site of acetylcholine esterase. The leaving group binds to the positive hydrogen of His 447 and breaks off the phosphate, leaving the enzyme phosphorylated. The regeneration of phosphorylated acetylcholine esterase is very slow and may take hours or days, resulting in accumulation of acetylcholine at the synapses. This inhibition causes convulsion, paralysis and finally death for insects and mammals [17] (Ragnarsdottir, 2000).

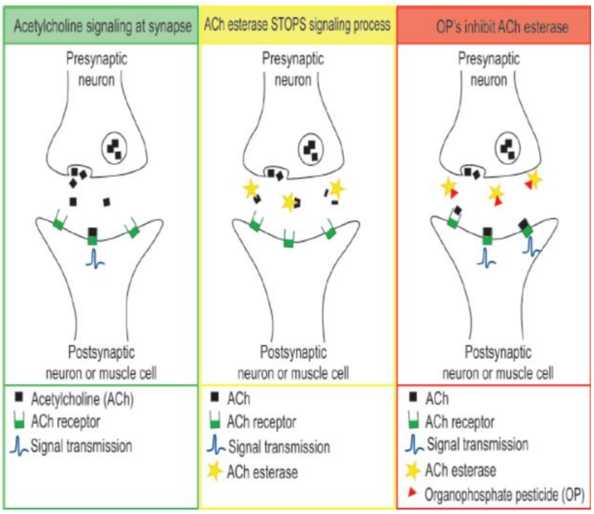

Figure 1 Organophosphate Compound Toxicity Mechanism

phenoxy groups [27] (Balali-Mood and Abdollahi, 2014). The mode of action of organophosphorus compounds involves inactivation of the enzyme acteylcholinesterase. Acetylcholine is a neurotransmitter that helps in the transmission of

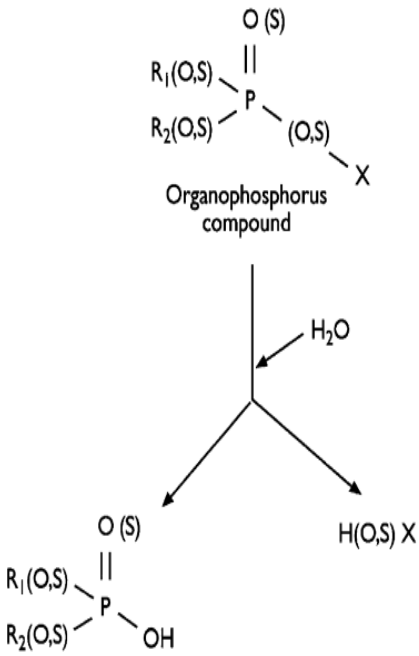

Figure 2 General Formula of Organophosphorus Compounds and Major Pathway of Degradation

3. Microbial degradation of pesticides

3.1 Types of microbes involved in degradation

Research on degradation of pesticide using microbes originated in 1940 and was based on aspects like pesticides with low toxicity, high efficiency and process of microbial degradation (Audus, 2021) [18]. Among the group of microorganisms, bacteria have been extensively found to degrade the pesticide residues, with low cost and environment friendly but the efficiency was relatively slow. Consequently, there are three main groups of microorganism in the soil, such as fungi, bacteria, and actinomycetes [26] (Singh, 2019) which are frequently involved in biodegradation, and some of the examples are mentioned in Table 2.

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Table 2 Examples of Microbes and the Pesticides Degraded by them

S. No	Group of Microbes	Species	Examples of Pesticides Degraded
1	Bacteria	Pseudomonas	Aldrin, chlorpyriphos, DDT, diazinon, hexachlorocyclohexane, endrin, parathion
		Bacillus	Chlorpyriphos, coumaphos, endrin, glyphoshate, methyl parathion, parathion, monocrotophos
		Alcaligenes	Chlorpyriphos, endosulphan
		Flavobacterium	Diazinon, glyphosate, parathion
2	Actinomycetes	Micromonospora, actinomyces, nocardia, streptomyces	Aldrin, carbofuran, chlorpyriphos, diuron, diazinon
3	Fungi	White rot fungi, Rhizopus, Cladosporium, Aspergillus fumigatus, Penicillium, Aspergillus, Fusarium, Mucor, Trichoderma spp, Mortirella sp.	Alachlor, endofuran, chlorpyriphos, lindane, malathion, esfenvelarate, fenitrooxon, DDT

3.2 Mechanism of microbial degradation of pesticides

Microbial degradation mainly involves the process of using pesticides which act as microbial nutrient, which gets broken down into small molecules, such as CO2 and H2O. This process is also known as enzymatic reaction as it involves the biochemical reactions using enzymes which would degrade the pesticide into smaller molecular compounds which are non-toxic (Tang, 2018) [19,33]. For example, Pseudomonas strain ADP used atrazine as the only carbon source, and three enzymes (AtzA, AtzB and AtzC) which were involved in the degradation of atrazine. The first enzyme catalyzed the reaction of hydrolysis dechlorination of atrazine to non-toxic hydroxyl atrazine, and it was a key enzyme of atrazine's biological degradation. The second enzyme catalyzed the dehydrochlorination of the hydroxy atrazine to produce N-isopropyl cyanuric amide. The third enzyme catalyzed the cyanuric acid and isopropylamine formated by N-isopropyl cyanuric amide. Finally, atrazine was degraded to CO2 and NH3 [20] (De Souza et al.,2019).

Three major steps in the microbial degradation involved:

Step 1: Adsorption of xenobiotic target on the surface of the cell membrane of microbe resulting into dynamic equilibrium process.

Step 2: The xenobiotic target got into the cell through the surface of the cell membrane whose efficiency and penetration rate were dependent on the molecular structure of the target.

Step 3: Rapid enzymatic reaction in the membrane due to the xenobiotic target compounds After these steps, one of the biological process involved in this process at the end is known as mineralization. Mineralization involved the conversion of organic compounds into inorganic compounds under the action of soil microbes. Mineralization resulted into complete degradation of pesticides into non-toxic inorganic substance. Another process known as Co-metabolism may also occur in which a chemical substance like insecticides, fungicides, and herbicides, which normally could be degraded not by bacteria or fungi, but by adding some organic matter such as exogenous or iso biomass as the primary energy [21] (Zhang et al., 2021).

3.3 Genetic aspect of microbial degradation of Pesticides

As per the research, pesticide degrading genes in microbes have been found to be located on

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plasmids, transposons, or on chromosomes. Recent studies have suggested that evolution of degradative pathways and the organization of catabolic genes, helps to develop genetically engineered microbes for degradation. Gene manipulation involves the design of engineered microorganisms who can deal with pesticides present at the contaminated sites. The simplest approach is to modify the degrading capabilities of existing metabolic pathways within an organism either by introducing additional enzymes from other organisms or by modifying the specificity of the catabolic genes already present. Catabolic genes responsible for the degradation of several xenobiotics, including pesticides, have been identified, isolated, and cloned into various other organisms such as Streptomyces, algae, fungi, etc. In addition, recombinant DNA technology have been successful in developing DNA probes that are being used to identify microbes that have unique ability to degrade pesticides isolated from the environmental niche [22].

3.4 Factors affecting Microbial Degradation of Pesticides

The effects of microbial degradation of pesticide were governed by internal factors and external environmental factors. Internal factors largely depend on the structure of pesticide and the nature of micro-organisms.

- Nature, metabolism and adaptation of Microbial species (internal factor): Research have shown that the nature of different or same species of microorganism had shown different effect on same organic substrate or toxic metal, and the microorganism had a strong ability to adapt environment. Through this adaptability, the new compounds could induce microorganism to produce the enzymes or establish a new enzyme system to degrade them [23].
- Structure of pesticide: The factors like molecular weight and spatial arrangement in the structure of pesticide, the number and type of substituents present in the structure of pesticides and location of pesticide affected the rate and efficiency of microbial degradation of pesticides. For instance, the polymeric compounds were less biodegradable than the low molecular weight compounds. The polymeric and complex structures of pesticides were more resistant to biodegradation, but simpler structure were degraded easily [24,34].
- Environmental factors: Temperature, humidity, salinity, pH, nutrition, carbon dioxide, oxygen, substrate concentration, surfactant, etc. normally affects the degradation. Bacteria or their enzymes needs a suitable temperature, pH and substrate concentration [25]. Temperature and humidity are the most important factors, affecting the growth and reproduction of bacteria [35-37] Zhu et al. in 2015 investigated that the degradation and mineralization of biaryl compounds in soil and compost by bacteria Ralstonia and Pickettii, and found that the non-ionic surfactants tween 80 can enhance bacteria's utilization of biaryl compounds under suitable soil moisture conditions, such as biphenyl, 4-chlorobipheny.

Conclusion and Future Aspects

There has been extensive research done on microbial degradation of pesticides resulting into identification of pesticide degrading microbial strains however the application of microbial bioremediation is limited, due to low efficiency and apt environment condition. The research shows that mineralization and co-metabolism have been important processes involved in the microbial degradation of pesticides. The factors that greatly influence the pesticide degradation are the molecular structure of pesticide, environment, solubility and its molecular orientation. Two groups of microbes greatly involved in degradation of pesticides are bacteria and fungi. The future prospects of microbial degradation of pesticides lies in the hands on genetic engineering and molecular biology. The approach will greatly focus on construction of recombinant bacteria. This can be done by transforming the gene of enzyme which will help in constructing the vector that will efficiently express the characteristics of degrading pesticide. The results of this approach will improve the expression level of specific proteins or enzymes, which will finally improve the efficiency of

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degradation. This will also overcome the problem that of stability of enzyme and will produce high enzyme activity. However, the only challenge to this approach is that the target strain and pesticide degradation products should be harmless and should not cause any environmental pollution.

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